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Aqueous extract of *Pterocarpus santalinoides* DC stem bark prevents L-NAME-induced hypertension in rat

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ABSTRACT

Background: *Pterocarpus santalinoides* stem bark is commonly used in Cameroonian medicine to treat many diseases including hypertension. Thus, this study was aimed to evaluate preventive effects of aqueous extract of *Pterocarpus santalinoides* (AEPS) stem bark on N^G-Nitro-L-arginine-methyl ester (L-NAME)-induced hypertension in rat. **Methods:** Normotensive rats received L-NAME (25 mg/kg intraperitoneally) concomitantly with AEPS (50, 100 and 200 mg/kg) or captopril (20 mg/kg) orally during 3 weeks. At the end of experimental period, arterial pressure and heart rate were recorded by invasive method. After sacrifice, blood, aorta and heart were harvested for biochemical analysis on homogenate. **Results:** Intraperitoneal injection of L-NAME induced in rat a significant increase ($p < 0.001$; $p < 0.01$; $p < 0.05$) of blood pressure, heart rate, malondialdehyde, total cholesterol, triglycerides, LDL-cholesterol, hepatic and renal markers functions. L-NAME also decreased significantly ($p < 0.001$; $p < 0.01$; $p < 0.05$) the levels of HDL-cholesterol, nitrites, glutathione, superoxide dismutase and catalase activities as compared to control rats. The AEPS prevented significantly the increase ($p < 0.001$) of hemodynamic parameters induced by L-NAME and various modifications of biochemical parameters (lipid profile, hepatic and renal markers functions) and oxidative stress markers evaluated. **Conclusion:** This study shows that the aqueous extract of *Pterocarpus santalinoides* prevents hypertension, dyslipidemia and oxidative stress induced by L-NAME in rat by attenuating endothelial dysfunction, liver and kidney's damages.

Keywords: L-NAME, *Pterocarpus santalinoides*, Hypertension, Oxidative stress, dyslipidemia.

INTRODUCTION

Hypertension is one of the most important factors associated with development of cardiovascular diseases such as stroke, heart and kidney failure, myocardial infarction, coronary and peripheral artery diseases [1]. Primary hypertension accounts for approximately 90 % of hypertensive patients and it is mainly caused by endothelial dysfunction which results from NO deficiency [2]. Hypertension is characterized by a high blood pressure and is defined as a sustained systolic blood pressure ≥ 140 mmHg or diastolic blood pressure ≥ 80 mmHg [3]. High blood pressure is particularly dangerous because it remains silent or asymptomatic, increases the risk of complications resulting to substantial morbidity and mortality. Several experimental models are used to demonstrate the antihypertensive effect of drug among which the administration of N^G-Nitro-L-arginine-methyl ester (L-NAME). L-NAME administrated to rats causes injury to the vascular endothelium and inhibits the biosynthesis of nitric oxide, a physiologically important vasodilator molecule which plays a major role in local circulatory control [4]. Treatments of hypertension are usually diuretics, beta and alpha blockers, angiotensin II receptors inhibitors, inhibitors of angiotensin-converting enzyme and mimetic nitric oxide [5]. Therefore in developing countries such as Cameroon, people resort to traditional medicine by phytotherapy to solve their health problems. However, scientific investigations are necessary to confirm these therapeutic claims and to regulate the use of herbal drugs by population [6]. *Pterocarpus santalinoides* (Fabaceae) is a plant used in traditional medicine to treat hemorroids, diarrhoea, inflammation disease, wounds and skin diseases [7, 8]. Phytochemical studies of *Pterocarpus santalinoides* stem bark revealed the presence of some bioactives metabolites such as flavonoids, alkaloids, saponins, phenols and tannins [9] which are known as cardioprotective compounds [10]. In addition, information provided by practitioners of traditional medicine in Centre Region of Cameroon indicates that the stem bark of *Pterocarpus santalinoides* (*P. santalinoides*) is used in hypertension management. The present study was designed to evaluate the preventive effects of the aqueous extract of *P. santalinoides* stem bark on L-NAME-induced hypertensive rats.

MATERIALS AND METHODS

Preparation of plant extract

Fresh *Pterocarpus santalinoides* (Fabaceae) stem barks were collected at Eseka (Centre Region of Cameroon) in May 2016. The plant was authenticated at the National Herbarium of Cameroon in comparison with a specimen registered under the No. HNC/42209. Fresh stem barks were dried at room temperature and reduced into powder. The powder sample (400 g) was decocted into 4 L of distilled water during 30 minutes following traditional healer's instructions. The filtrate obtained was dried at 45°C in drying-cupboard and the crude extract powder gave 37.4 g with 9.35 % of yield.

Animals

Wistar male rats, aged 10-12 weeks, weighing on average 200 g were used for this study. All animals were housed in the animal house of the Laboratory of Animal Physiology, University of Yaoundé I, Cameroon, and were maintained under ambient lighting (12h light-dark cycles) and temperature (25-28 °C). They had free access to water and food. All the procedures in this study followed the principles of laboratory animal use and care, and were approved by the Cameroon National Ethical Committee (authorization number FW-IRB00001954).

Chemicals

All chemicals used in this study were of analytical grade. N^G-Nitro-L-arginine-methyl ester (L-NAME) was supplied from Sigma Aldrich Chemical co (St. Louis, MO, USA) and captopril was obtained from Sandoz (Holzkirchen, Germany).

Induction of hypertension in rats

In order to evaluate preventive effects of *Pterocarpus santalinoides* on hypertension, L-NAME was administrated intraperitoneally at 25 mg/kg daily during 3 weeks [11]. The solution of L-NAME was obtained by solubilizing 5 g of L-NAME in 200 mL of saline (NaCl 9 %O). Forty-two rats were divided into six groups of seven rats each as follow: rats of group 1 received saline (10 mL/kg intraperitoneally), rats of group 2 received L-NAME (25 mg/kg intraperitoneally), rats of groups 3, 4, 5 and 6 received in addition to L-NAME, aqueous extract of *P. santalinoides* (50, 100 and 200 mg/kg) or captopril (20 mg/kg) respectively. Animals were daily treated and their weight was recorded every day for three consecutive weeks. At the end of this experimental period, arterial blood pressure and heart rate of all rats were measured by invasive method [12]. The rats were sacrificed under anesthesia by decapitation and the free running blood was collected. Afterwards abdominal fat, heart and left ventricle of rats were also dissected out and weighted.

Blood analysis

Total blood was centrifuged (3600 rpm for 15 min) and serum was collected for biochemical analysis of total cholesterol (Chol), triglycerides (TG), HDL-Cholesterol (HDL-Chol), albumin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine, urea, uric acid, K^+ and Na^+ ions levels using commercial diagnostic kits (Fortress, UK indication). Total proteins were obtained using the method of Gornall *et al.* [13] and LDL-Cholesterol (LDL-Chol) level was determined following the method described by Bilanda *et al.* [14].

Analysis of oxidative stress parameters in aorta and heart

The abdominal cavity was opened, aorta and heart were harvested. They were homogenized in Mc Even solution to prepare homogenate 20 %. Catalase and superoxide dismutase activities (SOD) were determined respectively according to Sinha [15] and Misra and Fridovish [16] methods, whereas malondialdehyde (MDA), reduced glutathione (GSH) and nitrites levels were assayed using respectively the procedures of Wilbur *et al.* [17], Ellman *et al.* [18] and Green *et al.* [19].

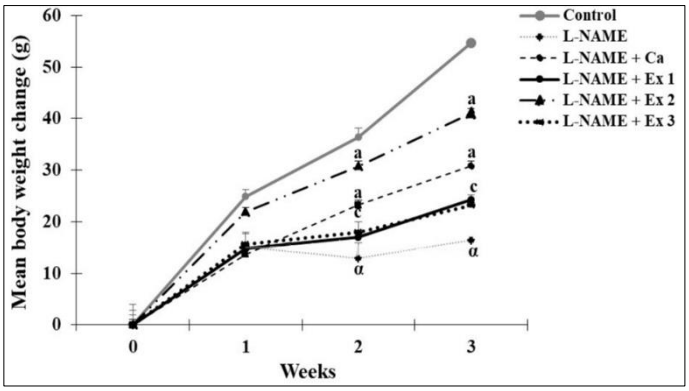
Statistical analysis

Data were expressed as mean ± S.E.M. Statistical significance was performed using one-way analysis of variance (ANOVA) followed by the Tukey post-test. A value of $p < 0.05$ was considered significant.

RESULTS

Effect of *Pterocarpus santalinoides* aqueous extract on weight gain in L-NAME-induced hypertensive rats

As shown in Figure 1, L-NAME administration induced a significant decrease ($p < 0.001$) of rat's body weight at weeks 2 and 3 respectively by 67.3 % and 69.9 % compared to normal control rats. The administration of the *P. santalinoides* aqueous extract at all doses prevented L-NAME lowering effect in body weight. The effect was more potent at the dose of 100 mg/kg. The plant extract (50, 100 and 200 mg/kg) inhibited the decrease of body weight by 47.5 % ($p < 0.01$), 148.2 % ($p < 0.001$) and 41.1 % ($p < 0.01$) at week 2; 27.4 % ($p < 0.05$), 139.3 % ($p < 0.001$) and 39.8 % ($p < 0.05$) at week 3 as compared to L-NAME group. Captopril in the same condition as the extract also prevented significantly the decrease ($p < 0.001$) of body weight as compared to L-NAME group.



Each point represents mean ± S.E.M. n = 7 rats per group. Ca: captopril 20 mg/kg; Ex 1, 2 and 3: Aqueous extract of *Pterocarpus santalinoides* (50, 100 and 200 mg/kg). $^{a}p < 0.001$ compared to control group. $^{c}p < 0.05$, $^{a}P < 0.001$ compared to L-NAME-induced hypertensive rats

Figure 1: Effect of *P. santalinoides* on body weight in L-NAME-induced hypertensive rats

Effect of *Pterocarpus santalinoides* aqueous extract on hemodynamic parameters in L-NAME-induced hypertensive rats

The effect of aqueous extract of *Pterocarpus santalinoides* on hemodynamic parameters are summarized in Table 1. A single daily administration of L-NAME for three weeks led to a significant increase ($p < 0.01$) of systolic blood pressure (SBP), mean blood pressure (MBP), diastolic blood pressure (DBP) and heart rate (HR) ($p < 0.05$) respectively by 62.5 %, 73.1 %, 90.1 % and 22.9 %.

Concomitant administration of L-NAME and plant extract (50, 100 or 200 mg/kg) and captopril significantly inhibited the increase of SBP respectively by 35.4 %, 34.1 %, 34.8 % and 30.2 %, of MBP by 37.1 %, 35.7 %, 38.9 % and 33.6 %, and of DBP by 40.5 %, 39.1 %, 45.1 % and 38.5 % in comparison to L-NAME group. At the same experimental conditions, captopril and the extract (50, 100 or 200 mg/kg) prevented significantly the increase of HR respectively by 12.5 % ($p < 0.05$), 11.6 % ($p < 0.05$), 20.2 % ($p < 0.01$) and 10.8 % ($p < 0.05$) compared to L-NAME-induced hypertensive rats.

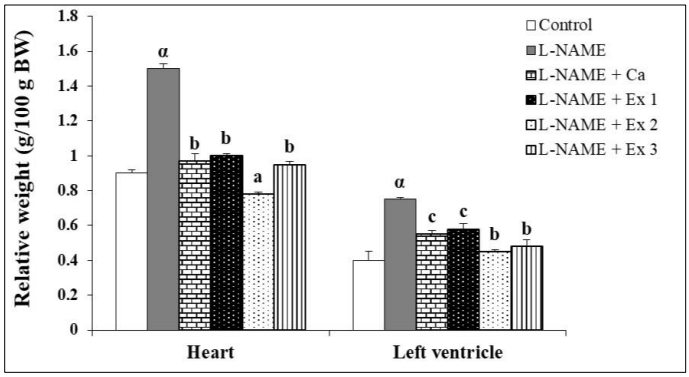
Table 1: Effect of *Pterocarpus santalinoides* on systolic blood pressure, mean blood pressure, diastolic blood pressure and heart rate in L-NAME-induced hypertensive rats

	Control	L-NAME	L-NAME + Ca	L-NAME + Ex 1	L-NAME + Ex 2	L-NAME + Ex 3
SBP (mmHg)	107.66 ± 3.98	175.00 ± 4.01$^{\alpha}$	122.20 ± 1.24^{a}	113.02 ± 5.66^{a}	115.38 ± 9.64^{a}	114.05 ± 5.40^{a}
MBP (mmHg)	90.02 ± 4.45	156.10 ± 4.74$^{\alpha}$	103.55 ± 11.46^{a}	98.20 ± 3.78^{a}	100.43 ± 8.70^{a}	95.31 ± 5.90^{a}
DBP (mmHg)	73.72 ± 5.65	140.09 ± 5.29$^{\alpha}$	86.15 ± 1.61^{a}	83.44 ± 2.80^{a}	85.34 ± 8.80^{a}	77.13 ± 6.01^{a}
HR (beat/min)	313.32 ± 4.88	385.05 ± 8.11$^{\beta}$	337.01 ± 7.73^{c}	340.38 ± 5.44^{c}	307.22 ± 8.62^{b}	343.63 ± 6.31^{c}

Each value represents mean ± S.E.M. n = 7 rats per group. Ca: captopril 20 mg/kg; Ex 1, 2 and 3: Aqueous extract of *Pterocarpus santalinoides* (50, 100 and 200 mg/kg). $^{\beta}p < 0.01$, $^{\alpha}p < 0.001$ compared to control group. $^{c}p < 0.05$, $^{b}p < 0.01$, $^{a}p < 0.001$ compared to L-NAME-induced hypertensive rats

Effect of *Pterocarpus santalinoides* aqueous extract on relative weight of heart and left ventricle in L-NAME-induced hypertensive rats

The administration of L-NAME during 3 weeks induced in rats a significant increase ($p < 0.001$) of heart and left ventricle relative weight respectively by 66.7 % and 87.5 % as compared to control rats (Figure 2). The aqueous extract of *Pterocarpus santalinoides* (50, 100 and 200 mg/kg) and captopril significantly prevented ($p < 0.05$, $p < 0.01$, $p < 0.001$) hypertrophy of heart and left ventricle induced by L-NAME respectively by 33.3 % and 22.7 %, 50.2 % and 40.1 %, 36.7 % and 36.1 %, and 35.3 % and 26.7 % as compared to L-NAME group.

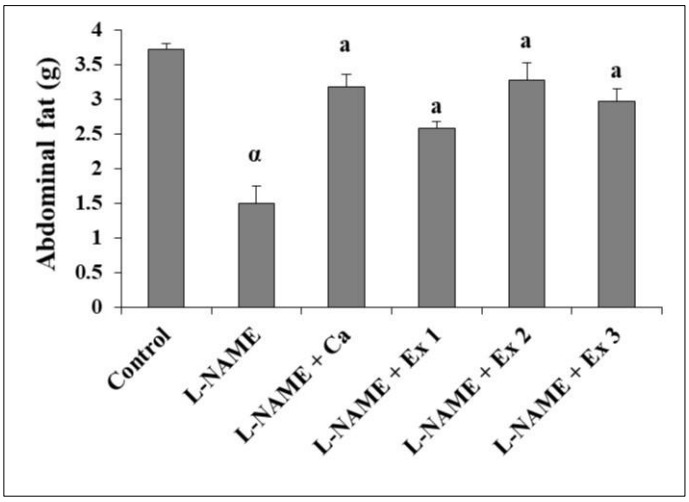


Each bar represents mean ± S.E.M. n = 7 rats per group. Ca: captopril 20 mg/kg; Ex 1, 2 and 3: Aqueous extract of *Pterocarpus santalinoides* (50, 100 and 200 mg/kg). $^{\alpha}p < 0.001$ compared to control group. $^{c}p < 0.05$, $^{b}p < 0.01$, $^{a}p < 0.001$ compared to L-NAME-induced hypertensive rats. BW: body weight

Figure 2: Effect of *Pterocarpus santalinoides* aqueous extract on relative weight of heart and left ventricle in L-NAME-induced hypertensive rats

Effect of *Pterocarpus santalinoides* aqueous extract on abdominal fat in L-NAME-induced hypertensive rats

Figure 3 shows that L-NAME administration induced in rats a significant decrease of abdominal fat's weight by 132.7 % ($p < 0.001$) as compared to normal control rats. *Pterocarpus santalinoides* at the doses of 50, 100 and 200 mg/kg and captopril significantly prevented ($p < 0.001$) the decrease of abdominal fats respectively by 73.4 %, 119.3 %, 99.3 % and 113.4 % in comparison to L-NAME-induced hypertensive rats.



Each bar represents mean ± S.E.M. n = 7 rats per group. Ca: captopril 20 mg/kg; Ex 1, 2 and 3: Aqueous extract of *Pterocarpus santalinoides* (50, 100 and 200 mg/kg). $^{\alpha}p < 0.001$ compared to control group. $^{a}p < 0.001$ compared to L-NAME-induced hypertensive rats

Figure 3: Effect of *Pterocarpus santalinoides* aqueous extract on abdominal fat in L-NAME-induced hypertensive rats

Effect of *Pterocarpus santalinoides* aqueous extract on lipid profile in L-NAME-induced hypertensive rats

The effect of *Pterocarpus santalinoides* aqueous extract on lipid profile in L-NAME-induced hypertensive rats is summarized in Table 2. Compared to control rats, administration of L-NAME for 3 weeks induced a significant increase ($p < 0.001$) of the levels of total cholesterol (Chol, 133.2 %), triglycerides (TG, 62.1 %), LDL-cholesterol (LDL-Chol, 137.9 %), atherogenic index (180.8 %) and a significant decrease ($p < 0.01$) of HDL cholesterol (HDL-Chol) by 13.7 %. The aqueous extract of *P. santalinoides* (50, 100 and 200 mg/kg) and captopril (20 mg/kg) significantly prevented ($p < 0.001$) dyslipidemia and the increase of atherogenic index induced by L-NAME in rats.

Table 2: Effect of *Pterocarpus santalinoides* on lipid profile in L-NAME-induced hypertensive rats

	Control	L-NAME	L-NAME + Ca	L-NAME + Ex 1	L-NAME + Ex 2	L-NAME + Ex 3
Chol (mg/dL)	121.51 ± 3.40	283.30 ± 6.45$^{\alpha}$	117.33 ± 5.82^{b}	139.70 ± 4.20^{b}	125.62 ± 3.10^{b}	128.67 ± 7.44^{b}
TG (mg/dL)	102.01 ± 0.78	165.32 ± 3.21$^{\alpha}$	93.89 ± 4.67^{b}	103.09 ± 7.01^{b}	98.56 ± 2.33^{b}	93.01 ± 4.70^{b}
LDL-Chol (mg/dL)	73.41 ± 0.67	174.60 ± 7.70$^{\alpha}$	86.70 ± 3.50^{b}	91.81 ± 1.20^{b}	95.10 ± 9.30^{b}	90.33 ± 2.62^{b}
HDL-Chol (mg/dL)	40.10 ± 8.82	34.56 ± 7.61µ	54.54 ± 2.43^{a}	40.90 ± 9.22^{c}	44.33 ± 1.50^{c}	54.22 ± 1.81^{a}
Atherogenic index	2.14 ± 0.14	6.01 ± 0.37$^{\alpha}$	1.86 ± 0.50^{a}	2.84 ± 0.40^{a}	2.76 ± 0.48^{a}	2.11 ± 0.14^{a}

Each value represents mean ± S.E.M. n = 7 rats per group. Ca: captopril 20 mg/kg; Ex 1, 2 and 3: Aqueous extract of *Pterocarpus santalinoides* (50, 100 and 200 mg/kg). $^{\mu}p < 0.05$, $^{\alpha}p < 0.001$ compared to control group. $^{c}p < 0.05$, $^{b}p < 0.01$, $^{a}p < 0.001$ compared to L-NAME-induced hypertensive rats

Effect of *Pterocarpus santalinoides* aqueous extract on some parameters of hepatic and renal functions in L-NAME-induced hypertensive rats

The effect of aqueous extract of *P. santalinoides* on hepatic and renal markers functions in L-NAME-induced hypertensive rats is presented in Table 3. The injection of L-NAME induced a significant increase in albumin, total protein, ALT and AST activities respectively by 56.2 % ($p < 0.001$), 56.5 % ($p < 0.001$), 38.5 % ($p < 0.01$) and 94.9 % ($p < 0.001$) in comparison to control rats. Concomitant administration of L-NAME with AEPS (50, 100 and 200 mg/kg) and captopril reduced significantly albumin level respectively by 37.8 % ($p < 0.05$), 43.5 % ($p < 0.05$), 26.5 % ($p < 0.05$) and 35.5 % ($p < 0.05$), total protein level respectively by 30.4 % ($p < 0.01$), 40.5 % ($p < 0.01$), 31.7 % ($p < 0.01$) and 35.8 % ($p < 0.01$), ALT and AST activities respectively by 68.1 % ($p < 0.001$) and 31.1 % ($p < 0.05$), 71.7 % ($p < 0.001$) and 39.5 % ($p < 0.05$), 68.1 % ($p < 0.001$) and 46.1 % ($p < 0.05$), 66.1 % ($p < 0.001$) and 53.7 % ($p < 0.001$) as compared to L-NAME group.

The effect of AEPS on renal markers functions was evaluated by the determination of urea, uric acid, creatinine, Na^+ and K^+ ions levels in the serum (Table 3). Rats receiving L-NAME exhibited a significant increase ($p < 0.001$) of the parameters mentioned above by 107.1 %, 46.4 %, 96.1 %, 137.4 % and 263.1 % as compared to control rats. Aqueous extract of *P. santalinoides* (50, 100 and 200 mg/kg) and captopril (20 mg/kg) prevented significantly the increase of urea respectively by 47.9 % ($p < 0.01$), 46.3 % ($p < 0.01$), 57.6 % ($p < 0.01$) and 49.6 % ($p < 0.01$), uric acid by 31.7 % ($p < 0.05$), 42.4 % ($p < 0.01$), 36.2 % ($p < 0.05$) and 30.4 % ($p < 0.05$), creatinine by 32.5 % ($p < 0.05$), 36.5 % ($p < 0.05$), 41.4 % ($p < 0.01$) and 47.4 % ($p < 0.01$), Na^+ and K^+ ions by 56.9 % ($p < 0.001$) and 43.5 % ($p < 0.01$), 61.1 % ($p < 0.001$) and 72.8 % ($p < 0.001$), 61.3 % ($p < 0.001$) and 60.9 % ($p < 0.001$), 46.2 % ($p < 0.01$) and 60.2 % ($p < 0.001$) comparatively to L-NAME group.

Table 3: Effect of *P. santalinoides* aqueous extract on some parameters of hepatic and renal functions in L-NAME-induced hypertensive rats

	Control	L-NAME	L-NAME + Ca	L-NAME + Ex 1	L-NAME + Ex 2	L-NAME + Ex 3
Hepatic markers						
ALT (U/L)	10.41 ± 2.40	35.33 ± 2.31$^{\alpha}$	10.90 ± 2.64^{a}	11.31 ± 2.72^{a}	10.23 ± 2.40^{a}	11.51 ± 2.72^{a}
AST (U/L)	65.90 ± 2.71	128.54 ± 1.20$^{\alpha}$	59.52 ± 6.53^{a}	88.50 ± 2.60^{c}	77.71 ± 4.62^{c}	69.30 ± 0.51^{c}
Albumin (mg/dL)	32.45 ± 0.20	52.21 ± 2.78$^{\alpha}$	32.74 ± 0.10^{c}	36.13 ± 0.52^{c}	32.88 ± 0.09^{c}	32.63 ± 1.22^{c}
Total protein (mg/dL)	9.09 ± 0.11	14.80 ± 1.21$^{\beta}$	9.53 ± 0.32^{b}	10.29 ± 0.70^{b}	8.82 ± 0.19^{b}	10.11 ± 1.10^{b}
Renal markers						
Urea (mg/dL)	44.70 ± 9.84	92.45 ± 2.33$^{\alpha}$	46.70 ± 1.11^{b}	48.33 ± 3.89^{b}	49.56 ± 1.44^{b}	39.22 ± 4.60^{b}
Uric acid (mg/dL)	4.67 ± 1.01	6.90 ± 1.60$^{\beta}$	5.89 ± 1.44^{c}	5.70 ± 1.40^{c}	4.80 ± 1.10^{b}	5.40 ± 1.22^{c}
Creatinine (mg/dL)	1.30 ± 0.03	2.54 ± 0.56$^{\alpha}$	1.30 ± 0.03^{b}	1.67 ± 0.44^{c}	1.62 ± 0.40^{c}	1.45 ± 0.44^{b}
Na^+ (mMol/L)	71.89 ± 1.33	170.70 ± 3.31$^{\alpha}$	91.81 ± 4.10^{b}	73.56 ± 3.09^{a}	66.67 ± 1.06^{a}	69.11 ± 0.70^{a}
K^+ (mMol/L)	2.02 ± 0.05	7.90 ± 1.51$^{\alpha}$	3.10 ± 0.67^{a}	4.45 ± 0.05^{b}	2.10 ± 0.33^{a}	3.10 ± 0.07^{a}

Each value represents mean ± S.E.M. n = 7 rats per group. Ca: captopril 20 mg/kg; Ex 1, 2 and 3: Aqueous extract of *Pterocarpus santalinoides* (50, 100 and 200 mg/kg). $^{\beta}p < 0.01$, $^{\alpha}p < 0.001$ compared to control group. $^{c}p < 0.05$, $^{b}p < 0.01$, $^{a}p < 0.001$ compared to L-NAME-induced hypertensive rats

***P. santalinoides* improves the oxidative state of the heart and aorta in L-NAME-induced hypertensive rats**

The administration of L-NAME during 3 weeks induced a significant decrease of catalase activity by 40.1 % ($p < 0.01$) in aorta and 24.8 % ($p < 0.05$) in heart as compared to control rats (Figure 4A). The aqueous extract of *P. santalinoides* prevented significantly the decrease of catalase activity in aorta by 85.2 % ($p < 0.001$) at 50 mg/kg, in aorta and heart respectively by 69.5 % ($p < 0.001$) and 39.4 % ($p < 0.01$) at 100 mg/kg, by 129.9 % ($p < 0.001$) and 22.9 % ($p < 0.05$) at 200 mg/kg, and by 155.1 % ($p < 0.001$) and 24.5 % ($p < 0.05$) in captopril group as compared to the hypertensive rats.

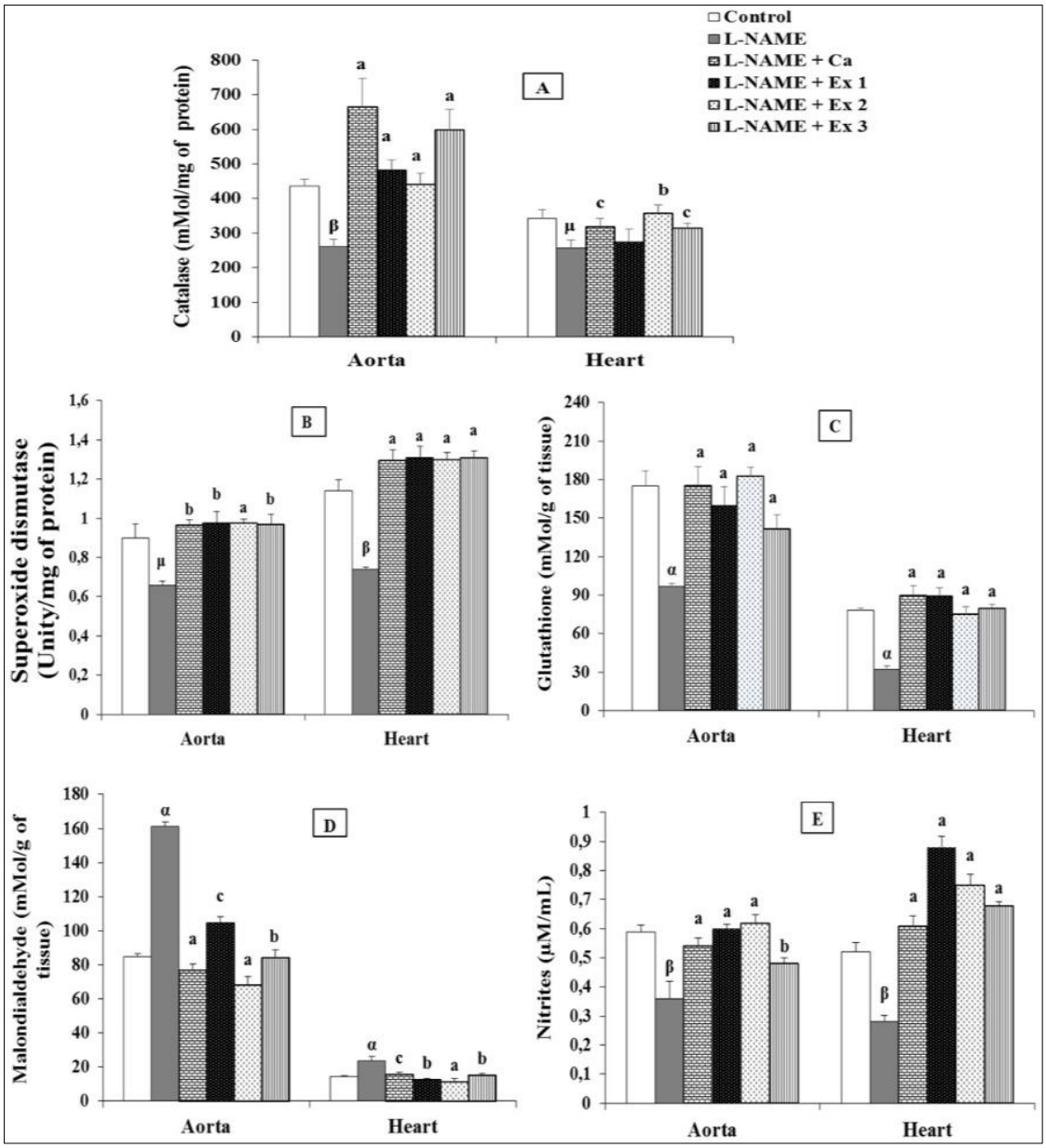
As shown in Figure 4B, the superoxide dismutase (SOD) activity of rats receiving L-NAME decreased significantly by 27.1 % ($p < 0.05$) in aorta and 35.1 % ($p < 0.01$) in heart as compared to control rats. The plant extract (50, 100 and 200 mg/kg) and captopril induced significantly the decrease of SOD activity respectively by 48.7 % ($p < 0.01$), 77.1 % ($p < 0.001$), 47.2 % ($p < 0.01$) and 46.7 % ($p < 0.01$) in aorta, and by 48.8 % ($p < 0.001$), 75.7 % ($p < 0.01$), 77.4 % ($p < 0.001$) and 75.1 % ($p < 0.001$) in heart comparatively to L-NAME group.

Figure 4C shows that L-NAME induced in rats a significant decrease of glutathione (GSH) level by 42.9 % ($p < 0.001$) in aorta and 59.2 % ($p < 0.001$) in heart when compared to control rats. Captopril

significantly increased ($p < 0.001$) GSH by 76.1 % and 182.4 % respectively in aorta and heart as compared to rats receiving L-NAME. The AEPS at the doses 50, 100 and 200 mg/kg prevented significantly ($p < 0.001$) the decrease induced by L-NAME respectively by 60.1 %, 80.2 % and 40.1 % in aorta and by 180.6 %, and 135.7 % and 150.5 % in heart as compared to hypertensive rats.

Daily administration of L-NAME during 3 weeks increased significantly malondialdehyde (MDA) level by 90.2 % ($p < 0.001$) in aorta and 64.5 % ($p < 0.01$) in heart as compared to control rats (Figure 4D). *P. santalinoides* (50, 100 and 200 mg/kg) and captopril induced a significant decrease ($p < 0.05$, $p < 0.01$, $p < 0.001$) of MDA respectively by 39.1 % ($p < 0.05$), 57.6 % ($p < 0.001$), 47.9 % ($p < 0.01$) and 52.4 % ($p < 0.001$) in aorta, and by 47.3 % ($p < 0.01$), 51.6 % ($p < 0.001$), 36.4 % ($p < 0.01$) and 33.1 % ($p < 0.05$) in heart comparatively to L-NAME group.

The effect of aqueous extract of *P. santalinoides* on endothelial function of L-NAME treated rats was determined by the measure of nitrites (NO_2^-) level in aorta and heart of rats (Figure 4E). The administration of L-NAME for 3 weeks leads to a significant decrease ($p < 0.01$) on NO_2^- levels in those organs respectively by 39.1 % and 46.2 % as compared to control rats. In comparison with animals receiving L-NAME, the administration of the plant extract at the doses 50, 100 and 200 mg/kg concomitantly with L-NAME prevented significantly the decrease in nitrites respectively by 66.7 % ($p < 0.001$), 72.2 % ($p < 0.001$) and 33.3 % ($p < 0.01$) in aorta, and by 214.3 % ($p < 0.001$), 167.9 % ($p < 0.001$) and 142.9 % ($p < 0.001$) in heart. The administration of captopril (20 mg/kg) reduced significantly ($p < 0.001$) nitrites level by 50.1 % in aorta and by 117.9 % in heart as compared to L-NAME-induced hypertensive rats.



Bars represent catalase activity (A), SOD activity (B), glutathione level (C), malondialdehyde level (D) and nitrites level (E). Data are expressed as mean ± S.E.M. n = 7 rats per group. Ca: captopril 20 mg/kg; Ex 1, 2 and 3: Aqueous extract of *Pterocarpus santalinoides* (50, 100 and 200 mg/kg). $^{\mu}p < 0.05$, $^{\beta}p < 0.01$, $^{\alpha}p < 0.001$ compared to control group. $^{c}p < 0.05$, $^{b}p < 0.01$, $^{a}p < 0.001$ compared to L-NAME-induced hypertensive rats

Figure 5: Effect of *P. santalinoides* aqueous extract on some markers of oxidative stress in L-NAME-induced hypertensive rats

DISCUSSION

The present study aimed to evaluate the preventive effect of *Pterocarpus santalinoides* stem bark aqueous extract on L-NAME-induced hypertensive rats. Intraperitoneally administration of L-NAME induced high blood pressure associated with left ventricle and heart hypertrophies. It is well known that intraperitoneally administration of L-NAME induced sustained hypertension [20]. In fact, L-NAME administration blocks the production by endothelial cells of nitric oxide a key regulator of cardiovascular system and metabolic homeostasis, which induced high blood pressure observed in hypertensive rats [21]. Aqueous extract of *Pterocarpus santalinoides* significantly prevented the increase of SBP, DBP, MBP and HR. Captopril, an enzyme conversion inhibitor is able to inhibit Angiotensin II production and low arterial pressure [22]. The antihypertensive effect of the plant extract might be mainly due to its ability to reduce the peripheral resistance through its vasodilation activity even though its effect on the renin-angiotensin system, which plays a pivotal role in the development of chronic L-NAME hypertension. Reducing this peripheral resistance will thus lead to a decrease in both systolic and diastolic blood pressures [23]. It is also well known that in this experimental model of arterial hypertension, the sympathetic system tone and baroreflex to phenylephrine are significantly increased [24]. The plant extract or captopril significantly reduced the weight of heart and left ventricle which was greater in L-NAME treated rats. Previous study showed that treatment of rats with L-NAME (25 mg/kg/day) during four weeks lead to ventricular hypertrophy, which hypertrophy is a compensatory response to chronic overloading in pressure or volume due to the growth of cardiomyocytes and synthesis of collagen after elevation of angiotensin and increase of cardiac weight [25]. The blockage of nitric oxide synthase by L-NAME causes dyslipidemia which plays an important role in hypertension pathogenesis [26]. Dyslipidemia observed in the present study is characterized by the increase of total cholesterol, LDL-cholesterol, triglycerides and the decrease of HDL-cholesterol level. We also noticed the elevation of atherogenic index which is associated with an increased risk of sudden cardiac death [27]. Captopril and *Pterocarpus santalinoides* improved lipid profile. These effects may be due to an increase in nitric oxide bioavailability and diverse bioactives compounds present in the extract such as flavonoids and saponines which possess vasorelaxant, antihypertensive and antihyperlipidemic activities [28].

In this study, L-NAME caused metabolic disorders including perturbation of hepatic enzymes and prooxidant/antioxidant balance in tissue. AST and ALT activities increased in hypertensive rats reflecting hepatic damages [29]. AEPS reduced significantly transaminases activities justifying that *P. santalinoides* protects liver against toxic effect of L-NAME. Intravenous administration of L-NAME in rats during 3 weeks increased urea, uric acid and creatinine levels. The enhancement of serum creatinine and urea are used as indicator of renal dysfunction [30]. The observed effects could be due to a renal failure, characterized by the impairment of glomerular filtration which induced the increase of urea, uric acid and creatinine concentrations in serum [31]. Aqueous extract of *Pterocarpus santalinoides* and captopril prevented significantly renal dysfunction and improved glomerular filtration in rats. The results obtained in the present study showed an increase of Na+ and K+ ions in L-NAME induced hypertensive rats, which would probably be explained by the generation of free radicals and the inhibition of Na^+-K^+ ATPase pump. The aqueous extract of *Pterocarpus santalinoides* significantly prevented the increase of K^+ and Na^+ levels, suggesting that the extract might interfere with the generation of free radicals and the inhibition of Na+- K+ ATPase pump. It has been reported that cardiac glycosides and phenols present in the extract stimulate the synthesis of the genes responsible of cellular regeneration of renal tissue [32]. This action could contribute to the AEPS effect on maintaining ions balance.

A significant decrease of reduced glutathione level, as well as SOD and catalase activities were observed in aorta and heart of L-NAME-induced hypertensive rats. The depletion of reduced glutathione is a consequence of oxidation of biological substrates. It is well known that inhibition of catalase and SOD activities induces accumulation of $H_20_2^-$ which exposes to oxidative stress [33]. The decrease of nitrites in L-NAME-induced hypertensive rats is causing by endothelial dysfunction [34]. Arterial hypertension is associated with elevation of reactive oxygen species (ROS) which impairs the bioavailability of NO [35]. Aqueous extract of *Pterocarpus santalinoides* leads to increase nitrites level, suggesting that the plant extract has a vasorelaxant effect. Malondialdehyde is considering like an index of lipid peroxidation due to the interaction of active oxygenates species with membranes fatty acids [36]. AEPS has a benefit effect in oxidative stress markers, suggesting that the plant extract is able to prevent lipid peroxidation induced by L-NAME and to enhance vasodilatation.

CONCLUSION

In conclusion, *Pterocarpus santalinoides* stem bark aqueous extract prevents hypertension, dyslipidemia, oxidative stress, hepatic and renal dysfunctions induced by L-NAME in rat. These effects could be due to its antioxidant activity and to its ability to increase nitric oxide bioavailability, a potent vasodilator agent. These effects validate the empirical uses of this plant in the treatment of cardiovascular disorders. Supplementary studies are necessary to characterize the active principle.

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